THE OCCURRENCE OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI ON TROPICAL FORAGE LEGUMES IN SOUTH FLORIDA

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ABSTRACT

A survey was conducted to quantify percentage root colonization, spore density, and the species distribution of vesicular-arbuscular mycorrhizal (VAM) fungi associated with cultivated tropical forage legumes in south Florida. Root and rhizosphere soil samples of 4 tropical forage legumes were collected from October 11 to 17, 1984, at 4 locations. Total spore density ranged from 5 to 679 per 100 g of air-dried soil, and the percentage root colonization varied from 3 to 41%. Legume species differed in percentage root colonization and total spore density among locations except for Desmodium heterocarpon *DC. cv. Florida (carpon desmodium), which showed little difference among locations in percentage root colonization. Fort Pierce had the highest total spore density for all legume species except* Macroptilium atropurpureum *Urb. cv. Siratro. Six species of VAM fungi were collected in this survey:* Gigaspora heterogama *Gerdemann & Trappe,* Gigaspora margarita *Becker & Hall,* Glomus etunicatum, *Becker & Gerdemann, Glomus intraradices Schenck & Smith,* Glomus *sp., and* Acaulospora spinosa *Walker & Trappe. The occurrence of VAM fungal species, as determined by spore numbers, was affected by the legume host and location.*

RESUMEN

Se realizó una encuesta para cuantificar el porcentaje de la colonización de raíces, densidad de esporas y la distribución de especies de vesiculo-arbuscular mycorrhiza (VAM) asociado con leguminosas forrajeras tropicales cultivadas en el sur de Florida. Muestras de raíces y suelos de la rizosfera de cuatro leguminosas forrajeras tropicales fueron coleccionadas del 11 al 17 de Octubre de 1984, en cuatro localidades. El rango de denisdad total de esporas fluctuó de 5 a 679/100 g de suelo secado al aire y el porcentaje de colonización de raíces varió de 3 a 41%. Las especies de leguminosas fueron diferentes entre localidades en cuanto a porcentaje de colonización de raíces y densidad total de esporas, con la excepción de Desmodium heterocarpon *DC. cv. Florida (carpon desmodium), el cual mostró poca diferencia entre localidades en el porcentaje de colonización de raíces. Fort Pierce tuvo la densidad total de espora más alta de todas las especies de leguminosa, excepto en* Macroptilium atropurpureum *Urb. cv. Siratro. Seis especies de hongos VAM fueron colectados en esta encuesta:* Gigaspora heterogama *Gerdemann & Trappe,* Gigaspora margarita *Becker & Hall,* Glomus etunicatum, *Becker & Gerdemann,* Glomus intraradices *Schenck & Smith,* Glomus *sp. y* Acaulospora spinosa *Walker & Trappe. La ocurrencia de las especies de hongos VAM, evaluada por el número de esporas, fue afectada por la leguminosa hospedante y la localidad.*

INTRODUCTION

There is widespread interest in the use of tropical forage legumes to increase production of tropical grasses in Florida's beef-cattle industry (Snyder *et al.* 1985). These legumes respond to inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi (Medina-Gonzales *et al.* 1987). However, before initiating a mycorrhizal inoculation program with these forage legumes in south Florida, a survey of the native populations of VAM fungi associated with the forage legumes, on a variety of soils, was needed.

Vesicular-arbuscular mycorrhizal associations have been observed in a wide variety of natural and agricultural ecosystems (Abbott and Robson 1977; Currah and Van Dyk 1986; Harley and Harley 1987). In Florida, the occurrence and distribution of VAM fungi in crops, including some tropical legumes (Schenck and Kinloch 1980; Schenck and Smith 1981), and sand-dune vegetation (Sylvia 1986), have been reported. However, there is no information on the degree of native VAM colonization of tropical forage legumes in Florida or on the susceptibility of different species of legumes to various genera and species of VAM fungi.

The objective of this survey was to obtain quantitative data on the amount of root colonization and the species distribution of VAM fungi associated with 4 cultivated tropical forage legumes from 4 different locations in south Florida.

MATERIALS AND METHODS

Root and rhizosphere soil samples of 4 tropical forage legumes were collected from October 11 to 17, 1984, at 4 locations in south Florida: Deseret Ranches, Deer park; Fort Pierce, Agricultural Research and Education Center (AREC); Ona, AREC; and Basinger Ranch, 109 Ranch (Fig. 1). Most of the soils belong to the order Spodosols. They are dominated by nearly level, somewhat poorly to poorly drained sandy soils with dark sandy subsoil layers. These soils are used primarily for pastures, vegetables, flowers, forestry, and citrus.

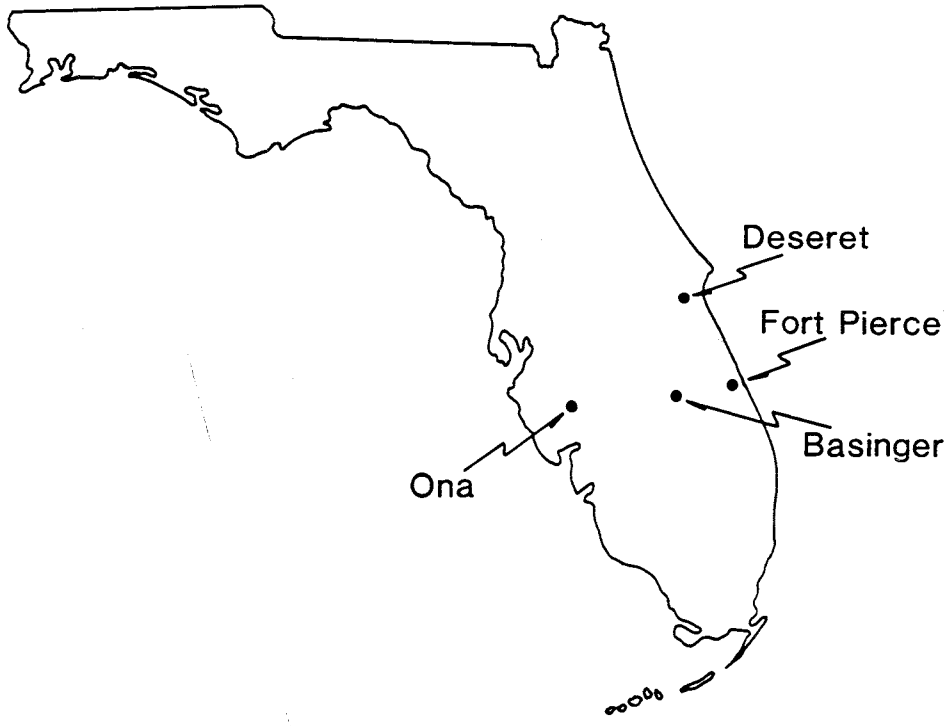

FIGURE 1
Collection sites for VAM fungi associated with 4 tropical forage legumes in south Florida.

The forage legumes sampled were: *Macroptilium atropurpureum* Urb. cv. Siratro, except at Deseret Ranches; *Aeschynomene americana* L. (commercial jointvetch); *Vigna adenantha* Marechal, Mascherpa and Stainier; and *Desmodium heterocarpon* DC. cv. Florida (carpon desmodium). The legumes were mixed with pasture grasses at

the time of sampling. Three samples of soil plus roots were collected to a depth of 15 cm for each legume stand at each location. Samples, consisting of 3 subsamples of approximately 1.5 kg, were placed in plastic bags and transported to the laboratory on the same day.

Samples were sieved through a 4-mm screen, and 100 g subsamples were removed and stored at 5°C for spore extraction. Legume roots were separated manually from grass roots. A 0.5 g portion of each root sample was cleared in 10% KOH and stained with trypan blue in lactophenol (Kormanik and McGraw 1982). Root colonization by VAM fungi was estimated by the gridline-intersect method of Giovannetti and Mosse (1980).

Chemical content of a composite soil sample from each location was determined by the Soil Testing Laboratory, University of Florida (Rhue and Kidder 1984). Mehlich-I solution (0.05 *M* KCl + 0.013 *M* H_2SO_4) was used to extract Al, Ca, K, Mg and P. All elements were analyzed in the filtrate by atomic absorption spectrophotometry, except P which was determined by the ammonium molybdate/ascorbic acid colorimetric method. Soil pH was determined on a 1:2 (v/v) soil:water suspension. Organic matter was estimated by oxidation with 1 *N* $K_2Cr_2O_7$ in the presence of H_2SO_4 (Rhue and Kidder 1984).

Spores of VAM fungi were collected from soil by the wet sieving method of Daniels and Skipper (1982) on 425, 90 and 45 μm-mesh sieves. Fractions retained on 90 and 45 μm-mesh sieves were centrifuged (3000 rpm) for 3 min in water. The pellet was resuspended in 40% sucrose solution and centrifuged for 1.5 min. Spores were identified to species where possible (Schenck and Smith 1982; Trappe 1982). In addition, spores or washed roots were placed in pasteurized Arrendondo loamy sand soil in 15-cm diameter plastic pots in the greenhouse and planted with bahiagrass (*Paspalum notatum* Flugge), *Desmodium heterocarpon*, or *Macroptilium atropurpureum* in an attempt to isolate VAM fungi in a similar manner to that described by Gerdemann and Trappe (1974) as the "inoculated pot culture" method.

RESULTS AND DISCUSSION

Results of soil pH and chemical analysis of soil samples reflect the different management regimes, including lime and fertilizer, at each location (Table 1).

Total spore density at the 4 locations ranged from 5 to 679 per 100 g of air-dried soil and percentage root colonization varied from 3 to 41%. Miller *et al.* (1979) observed variable degree of mycorrhizal root colonization (4 to 74%) in forage grasses and legumes in Brazil. Except for *D. heterocarpon*, legume species differed in percentage root colonization and total spore density among locations (Table 2). Fort Pierce had the highest total spore density for each legume species except for *M. atropurpureum*.

Attempts were made to relate percentage root colonization and total spore density to soil P and other soil chemical characteristics, but no significant relationships were found. Abbott and Robson (1977) and Hayman (1978) also reported that spore numbers were not correlated with soil P or soil pH in cultivated soils.

There was a significant ($P < 0.05$) correlation between root colonization and total spore density for all legume species at Basinger ($r = 0.70$) and Deseret Ranches ($r = 0.76$), but not at Fort Pierce and Ona. Giovannetti (1985) and Miller *et al.* (1976) reported a correlation between root colonization and spore density, while Hayman and Stovold (1979) and Giovannetti and Nicolson (1983) found no correlation. This apparent discrepancy may be due to different sampling methods; Giovannetti (1985) collected samples within the same plant species and sites, while the other researchers collected samples from many different plant species and sites.

Spore production and root colonization are influenced by seasonal variations (Giovannetti 1985; Sylvia 1986), host plant and stage of development (Saif and Khan 1975; Schenck and Kinloch 1980), and soil type (Lopes *et al.* 1983). In this survey

there was only one sampling time, so it was not possible to separate seasonal or host developmental effects on root colonization and total spore density.

The 6 species of VAM fungi collected in this survey were: *Gigaspora heterogama* Gerdemann & Trappe, *Gigaspora margarita* Becker & Hall, *Glomus etunicatum* Becker & Gerdemann, *Glomus intraradices* Schenck & Smith, *Glomus* sp. and *Acaulospora spinosa* Walker & Trappe. The unidentified *Glomus* sp. was dark brown to black, 200-250 μm in diam, and had a single wall of 8-14 μm thickness.

TABLE 1

Chemical characteristics of the soils sampled for VAM fungi associated with 4 tropical forage legumes at 4 locations in south Florida.

Location	Legume[1] species	O.M.[2]	pH	Soil nutrient levels				
				Al	Ca	Mg	K	P
		(%)		(mg/kg soil)				
Fort Pierce	AA	1.4	6.0	23.2	314.4	93.2	7.6	4.4
	DH	1.2	5.3	22.0	241.0	15.2	16.8	4.6
	VA	1.3	5.5	25.2	242.4	21.2	20.8	4.0
	MA	1.3	5.2	62.0	270.0	25.6	13.6	16.8
Ona	AA	3.4	6.1	44.4	1320.0	143.6	64.0	23.2
	DH	3.1	5.4	36.8	920.0	120.4	29.6	8.0
	VA	2.5	4.9	22.8	480.0	70.4	46.0	7.6
	MA	5.7	4.7	55.2	800.0	95.6	43.6	5.6
Deseret	AA	2.5	6.1	66.8	780.0	67.6	40.4	6.0
	DH	2.3	6.0	27.6	1040.0	94.8	27.6	4.0
	VA	2.8	7.2	30.4	1600.0	141.2	55.2	9.0
Basinger	AA	4.7	5.3	28.4	960.0	32.0	28.0	4.0
	DH	3.5	5.2	26.4	460.0	100.8	46.0	7.0
	VA	3.6	5.1	28.4	540.0	111.6	58.4	8.0
	MA	4.2	5.2	36.4	640.0	129.6	94.0	11.0

[1]AA = *Aeschynomene americana*; DH = *Desmodium heterocarpon*; VA = *Vigna adenantha*; MA = *Macroptilium atropurpureum*.
[2]Organic matter content based on a composite of 3 samples for each legume.

TABLE 2

Mean percentage of root colonization and total spore density of VAM fungi among forage legumes at 4 locations in south Florida. Values represent the mean of 3 samples.

Location	Root colonization	Total spore density
	(%)	(spores/100 g soil)
	Aeschynomene americana	
Ft. Pierce	7	302
Ona	6	161
Basinger	5	8
Deseret	30	146
	Desmodium heterocarpon	
Ft. Pierce	12	679
Ona	15	377
Basinger	12	19
Deseret	16	12
	Vigna adenantha	
Ft. Pierce	5	535
Ona	41	77
Basinger	20	26
Deseret	25	36
	Macroptilium atropurpureum	
Ft. Pierce	15	23
Ona	8	295
Basinger	3	5

TABLE 3

Mean spore numbers of VAM fungal species assoicated with 4 forage legumes at 4 locations in south Florida. Values represent the mean of 3 samples.

Location	VAM fungi[1]					
	GM	GH	EHU	INT	AS	GS
			Aeschynomene americana			
Ft. Pierce	141	66	22	43	0	16
Deseret	10	136	0	0	0	0
Basinger	0	8	0	0	0	0
Ona	0	16	29	115	0	0
			Desmodium heterocarpon			
Ft. Pierce	2	114	255	307	0	0
Deseret	2	9	0	0	0	0
Basinger	0	0	19	0	0	0
Ona	0	0	325	51	0	0
			Vigna adenantha			
Ft. Pierce	1	39	252	241	0	0
Deseret	21	15	0	0	0	0
Basinger	0	0	25	0	0	0
Ona	28	0	28	11	10	0
			Macroptilium atropurpureum			
Ft. Pierce	0	11	0	4	0	8
Basinger	0	5	0	0	0	0
Ona	0	0	40	254	0	0

[1]GM = *Gigaspora margarita*; GH = *Gigaspora heterogama*; *ETU* = *Glomus etunicatum*; INT = *Glomus intraradices*; AS = *Acaulospora spinosa*; GS = *Glomus* sp.

The occurrence of VAM fungal species, as determined by spore numbers, associated with all forage legumes at the 4 locations is presented in Table 3. The maximum number of spores of *G. margarita* occurred at Fort Pierce associated with *A. americana.* Spores of *G. margarita* were not found associated with *M. atropurpureum* at any of the 4 locations. Spores of *G. heterogama*, *G. etunicatum*, and *G. intraradices* were found associated with all legumes, in at least one of the locations. *Glomus heterogama* occurred in greatest numbers at Deseret Ranches and Fort Pierce assoicated with *A. americana* and *D. heterocarpon*, respectively. The maximum number of spores of *G. etunicatum* occurred at Ona and Fort Pierce associated with *D. heterocarpon.* A high number of spores of *G. etunicatum* was also found at Fort Pierce associated with *V. adenantha. Glomus intraradices* was recovered in greater numbers from *D. heterocarpon* and *V. adenantha* at Fort Pierce, as well as from *M. atropurpureum* at Ona. The unidentified *Glomus* sp. occurred in lower numbers than the other 2 species of *Glomus*; it was only found at Fort Pierce, associated with *A. americana* and *M. atropurpureum. Acaulospora spinosa* was only recovered from *V. adenantha* at Ona.

Overall root colonization by VAM fungi was low (most values below 20%) which indicates that (1) the native population of VAM fungi is not very infective and (2) field inoculation may be effective.

Attempts to establish pot cultures of VAM fungi recovered in this survey were only successful with *G. etunicatum* and *G. intraradices.* These 2 fungi were shown to be effective in increasing the growth of several forage legumes (Medina-Gonzales *et al.* 1987) and are being evaluated for use as field inoculants for forage legumes in P-deficient soil.

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